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Abstract

Level of humoral immune response to GAT has been associated with the MHC of chickens. Matings between two unrelated lines of chickens with low antibody response to GAT (G-B1 and S1 Line *B*19L) resulted in progeny that were higher responders to GAT challenge ($P < .05$) than either of the parental lines. Progeny of matings between two related sublines that are low responders to GAT (S1 Lines *B*19L and *B*1L) had antibody responses to GAT that were not higher than the parental lines. Progeny of the between-line cross were backcrossed to S1 *B*19L and G-B1 (*B*13) parental lines, as well as mated *inter se.* These matings produced F2 progeny whose GAT response was significantly associated ($P < .05$) with their MHC (*Ea-B*) type. The progeny were of three MHC types (*B*19*B*19, *B*19*B*13, and *B*13*B*13) that bound 66.6, 71.9, and 4.6%, respectively, of the GAT in a radioimmunoassay. The results from these matings suggest that MHC or MHC-linked genes, as well as genes not linked to the MHC, contribute to control of humoral immune response to GAT in the lines of chicken tested.

Keywords

gene complementation, humoral immune response, G-B1 line, major histocompatibility complex, chickens

Disciplines

Agriculture | Animal Sciences | Genetics | Poultry or Avian Science

Comments

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Gene Complementation in Biological Crosses for Humoral Immune Response to Glutamic Acid-Alanine-Tyrosine[1]

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ABSTRACT Level of humoral immune response to GAT has been associated with the MHC of chickens. Matings between two unrelated lines of chickens with low antibody response to GAT (G-B1 and S1 Line B^{19L}) resulted in progeny that were higher responders to GAT challenge ($P < .05$) than either of the parental lines. Progeny of matings between two related sublines that are low responders to GAT (S1 Lines B^{19L} and B^{1L}) had antibody responses to GAT that were not higher than the parental lines. Progeny of the between-line cross were backcrossed to S1 B^{19L} and G-B1 (B^{13}) parental lines, as well as mated *inter se*. These matings produced F_2 progeny whose GAT response was significantly associated ($P < .05$) with their MHC (*Ea-B*) type. The progeny were of three MHC types ($B^{19}B^{19}$, $B^{19}B^{13}$, and $B^{13}B^{13}$) that bound 66.6, 71.9, and 4.6%, respectively, of the GAT in a radioimmunoassay. The results from these matings suggest that MHC or MHC-linked genes, as well as genes not linked to the MHC, contribute to control of humoral immune response to GAT in the lines of chicken tested.

(*Key words*: gene complementation, humoral immune response, G-B1 line, major histocompatibility complex, chickens)

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INTRODUCTION

In mice, the *I* region genes of the *H-2* complex (MHC) control humoral immune response to some antigens (such as GAT), as well as the expression of Ia antigens (Shreffler and David, 1975). Benedict *et al.* (1975) described the close association between immune response to GAT (Ir-GAT) and the chicken *B* complex (MHC). However, it has not been possible to associate differences in *B-L* (the chicken homolog of the mouse *I* region) with differences in humoral immune response to GAT in chickens. Restriction fragment length polymorphisms generated by probing DNA from high and low antibody responders to GAT (Ir-GAT$^{H \text{ or } L}$) with a Class II (*B-L* β) probe were not associated with Ir-GAT differences (Pitcovski *et al.*, 1989).

Although Ir-GAT is associated with the chicken MHC, it is not readily observable what relationship connects high or low antibody response to the MHC. Whatever the mechanism, caution should be exercised in comparing mouse immune response to mechanisms of chicken immune response. The limited antigen diversity of linear amino acid polymers (such as GAT) ensures that most inbred mouse lines either respond or do not respond when challenged (Benacerraf and Dorf, 1977). The contrast in humoral immune response in chickens is not one of absolutes but one of degree. Chickens of the MHC alleles tested to establish genetic associations were either low or high responders to GAT (Benedict *et al.*, 1975) but not non-responders.

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Matings were designed to investigate the genetic control of humoral immune response in chickens. Low responder lines of chickens were crossed to produce progeny that could be assayed for evidence of gene complementation and MHC effects that would result in high antibody response to GAT.

MATERIALS AND METHODS

Experimental Animals

Pevzner *et al.* (1978) reported evidence of recombination between the serologically detectable MHC gene products (*B-F* and *B-G*) and the gene or genes responsible for immune response to GAT (Ir-Gat) in the S1 line of chickens. Since then, the S1 line has been maintained as four sublines with forced segregation for homozygous MHC type (B^1 or B^{19}) and high or low humoral immune response ($Ir\text{-}Gat^H$ or $Ir\text{-}GAT^L$) to challenge by immunization with the linear amino acid polymer GAT. The inbreeding coefficient for the four sublines was approximately .52 (Nordskog and Cheng, 1988). Chickens were housed in conventional floor pens or wire cages and consumed feed meeting National Research Council (1984) requirements and water *ad libitum*. All chickens received vaccinations for Marek's disease and fowl pox.

Chickens of the highly inbred [greater than 99%, Knudtson and Lamont (1989)] G-B1 lines were also used. The MHC haplotypes B^{13} and B^6 are represented by G-B1 and G-B2, respectively, and can be considered congenic, differing only by MHC type. The G-B1 and G-B2 lines have been widely used in immunogenetic studies by several laboratories (Miggiano *et al.*, 1976; Pink and Miggiano, 1977; Morrow and Abplanalp, 1981; Maccubbin and Schierman, 1986).

Complementation Matings

Two types of crosses were made to test for gene complementation between low GAT-responder sublines. One cross was between B^{19} $Ir\text{-}GAT^L$ and B^1 $Ir\text{-}GAT^L$ chickens of the S1 line. The other type of cross was between S1 line B^{19} $Ir\text{-}GAT^L$ chickens and unrelated G-B1 (B^{13}) chickens, also low responders to GAT challenge (E. Steadham, unpublished data).

Backcrosses to parental sublines and *inter se* matings were made with the G-B1 × S1 F_1 roosters and hens. These progeny were MHC (*B-G* and *B-F*) typed by hemagglutination at 6 wk of age.

Glutamic Acid-Alanine-Tyrosine Immunization Challenge

Birds were challenged with GAT beginning at 12 wk of age, and their immune sera were assayed by GAT radioimmunoassay. The GAT[3] used in all phases of research was from the same manufacturing lot. On Day 1 of the immunization protocol, each chicken received 1 mL of antigen emulsion, .5 mL i.m. in each breast. The emulsion was one part complete Freund's adjuvant: one part GAT solution (500 μg GAT/mL of .01 *M* PBS, pH 7.5), for a total dose of 250 μg GAT per bird. On Day 21 a second dose of antigen (.5 mL of 500 μg GAT/mL in .01 *M* PBS, pH 7.5) was administered i.v. The chickens were bled on Day 28 to measure secondary response, and the sera were collected and frozen until assayed.

Glutamic Acid-Alanine-Tyrosine Radioimmunoassay

The GAT was labeled with $Na^{125}I$[4] by the chloramine-T method (Hunter, 1973) with modifications of Pevzner *et al.* (1978). A modified Farr radioimmunoassay (Bluestein *et al.*, 1971) was used to determine antigen binding by immune serum. The labeled GAT was diluted 1:1000 in assay buffer (.01 *M* PBS with 1% normal chicken serum). Antisera were diluted 1:10 in assay buffer. Normal chicken serum (NCS) was diluted and used for positive and negative controls. For the assay, 25 μL of the labeled GAT dilution was mixed with 25 μL of each antiserum dilution in a 1.5-mL microcentrifuge tube. The primary reaction was incubated for 1 h at 4 C. After the incubation, 50 μL of rabbit anti-chicken IgG (prepared in-house) was added to the

[3]Miles Laboratories, Elkhart, IN 46515.
[4]IMS.300, Amersham Corp., Arlington Heights, IL 60005.

TABLE 1. Secondary humoral immune response to GAT parental lines by *Ea-B* and Ir-GAT type as determined by radioimmunoassay

Line	*Ea-B*	Ir-GAT[1]	n	Percentage GAT bound ($\bar{x}$ ± SEM)
S1	$B^{19}B^{19}$	High	23	80.3 ± 3.4[a]
S1	$B^{19}B^{19}$	Low	25	27.4 ± 5.6[c]
S1	$B^{1}B^{1}$	High	19	88.7 ± 3.3[a]
S1	$B^{1}B^{1}$	Low	23	35.2 ± 6.2[bc]
G-B1	$B^{13}B^{13}$	Low	18	5.3 ± 1.1[d]
G-B2	$B^{1}B^{1}$	High	20	43.0 ± 3.2[b]

[a-d]Means (± SEM) within a column with no common superscripts differ significantly ($P < .05$).

[1]Ir-GAT = phenotypic immune response to GAT.

TABLE 2. Secondary humoral immune response to GAT challenge of F_1 birds as determined by radioimmunoassay

Line and MHC haplotype				n[1]	Percentage GAT bound ($\bar{x}$ ± SEM)
Sire		Dam			
S1	B^{1L}	S1	B^{19L}	40	27.1 ± 4.4
S1	B^{19L}	S1	B^{1L}	69	18.4 ± 2.7
G-B1	B^{13}	S1	B^{19L}	82	53.2 ± 3.4
S1	B^{19L}	G-B1	B^{13}	57	48.2 ± 3.6

[1]Number of progeny tested by GAT challenge.

samples and the negative control (antigen dilution plus NCS dilution) tubes. Positive control tubes (antigen dilution plus NCS dilution) received 50 μL of 20% trichloroacetic acid (TCA). The samples were incubated for 1 additional h at 4 C. After the second incubation, the samples were centrifuged (10 min in a microcentrifuge) and a 50 μL sample of supernatant was removed for assay on a gamma counter. Results were expressed as the percentage labeled antigen (GAT) bound by the immune serum dilution by using the formula:

$$\text{percentage GAT bound} = 100 - \left(\frac{(\text{experimental count} - \text{TCA count})}{(\text{NCS count} - \text{TCA count})}\right) \times 100.$$

Statistical Analysis

Analyses of variance by the General Linear Models procedure of SAS® software (SAS Institute, 1985) were performed on assay results by using the percentage GAT bound as the variable and the traits under consideration (e.g., *Ea-B*, Ir-GAT) as sources of variation. Differences between means were tested by Duncan's multiple range test or by *t* test.

RESULTS

The parental subline mean percentage GAT bound by immune serum collected after the second GAT immunization is shown in Table 1. Table 2 shows the mean percentage GAT bound by secondary immune sera from F_1 chickens. Two types of crosses were made to produce F_1 progeny. The first type of cross was the within-line cross of B^{1L} chickens with B^{19L} chickens. These sublines share the same Ir-GAT phenotype and are derived from the same background but differ at the MHC. The second type of cross was between unrelated lines, G-B1 and S1 line B^{19L} chickens. Within mating type, progeny did not differ in GAT response by MHC type of the sire. The mean percentage GAT bound of the F_1 birds of the S1 within-line matings did not differ from the GAT bound by the parental S1 sublines. The F_1 progeny means of the S1 × G-B1 between-line crosses did not differ by sire MHC type, but were significantly higher ($P < .05$) than the mean percentage GAT bound by the corresponding parental sublines.

The between-line cross (S1 × G-B1) progeny were backcrossed to each parental subline (G-B1 and S1 B^{19L} chickens) and also mated *inter se* to produce an F_2 population. The resulting progeny were of three different *Ea-B* types (homozygous B^{19}, homozygous B^{13}, and heterozygous $B^{19}B^{13}$). Table 3 shows the mean percentage GAT bound by secondary immune serum by F_2 *Ea-B* type. Regardless of their derivation, the chickens that were B^{13} homozygotes produced low levels of antibody after challenge with GAT.

Table 4 shows the ANOVA for GAT response by mating type [F_1 backcrossed to S1 B^{19L} roosters or hens, F_1 backcrossed to G-B1 roosters or hens, and F_1 ($B^{19}B^{13}$) crossed with F_1]. Individual sire was a significant ($P < .001$) source of variation

TABLE 3. Secondary humoral immune response to GAT of F_2 birds produced from backcross and *inter se* matings as determined by radioimmunoassay

Ea-B of progeny	n	Percentage GAT bound
		($\bar{x}$ ± SEM)
$B^{19}B^{19}$	95	66.6 ± 3.2[a]
$B^{19}B^{13}$	195	71.9 ± 2.5[a]
$B^{13}B^{13}$	96	4.6 ± 1.4[b]

[a-b]Means within a column with no common superscripts differ significantly ($P < .05$).

for GAT response of progeny only from S1 B^{19L} backcrosses. Progeny produced from $F_1 \times F_1$ and G-B1 $\times F_1$ matings significantly differed ($P < .0001$) in GAT response based on their *Ea-B* type.

Sires used in crosses between S1 and F_1 were of two *Ea-B* types ($B^{19}B^{19}$ from S1 or $B^{19}B^{13}$ from F_1), and sires crossed between G-B1 and F_1 were of two *Ea-B* types ($B^{13}B^{13}$ from G-B1 or $B^{19}B^{13}$ from F_1). In crosses of F_1 and S1 B^{19L}, the sire *Ea-B* type (which also represents the sire line) was a significant ($P < .001$) source of variation in GAT response of their progeny (Table 5). The progeny of $B^{19}B^{19}$ sires had a higher GAT response than the progeny of $B^{19}B^{13}$ sires (data not shown). When sire *Ea-B* (line) type was included in the analysis, the *Ea-B* type of the $F_1 \times$ S1 progeny was also a significant source of variation; heterozygous progeny ($B^{19}B^{13}$) bound a lower percentage of GAT than the homozygous ($B^{19}B^{19}$) progeny (data not shown).

DISCUSSION

Matings between two unrelated low GAT-responder sublines of chickens resulted in progeny that had higher antibody responses to GAT challenge than either of the parental lines, suggesting that gene complementation was occurring. The F_1 progeny produced from within-line crosses (S1-$B^{19L} \times B^{1L}$) were low antibody responders to GAT challenge. The progeny produced by crossing the F_1 chickens to S1 line B^{19L} chickens or G-B1 chickens and mating *inter se* showed an association of immune response to GAT with progeny *Ea-B* type.

Genes not linked to the MHC were responsible for the complementation in GAT response seen in F_1 progeny from the between-line crosses. Dorf *et al.* (1974) presented evidence that nonresponse or response in some inbred mouse lines was due to genes within or linked to the mouse MHC and that quantitative variation in response was mediated by non-MHC genes. This may be one cause of what is occurring after chickens are challenged with GAT. The G-B1 chickens may be true nonresponders to GAT (the assay variation limits the ability to discriminate between no response and low response), but they may carry complementary genes that allow B^{19L} chickens (that are low responders, not nonresponders) to produce progeny that are high antibody responders to GAT. The crosses within the S1 line would not have introduced the complementary alleles that would result in high GAT response if those alleles were

TABLE 4. Analyses of variance for percentage GAT bound by progeny groups produced from backcross and *inter se* matings

	F_1 birds crossed					
	to S1 B^{19L}		to G-B1 B^{13}		*Inter se*	
Source of variation	df	MS	df	MS	df	MS
Sire	9	2,972***	10	127	7	405
Progeny *Ea-B*	1	2,133	1	207,707****	2	75,529****
Dam(Sire)	4	278	8	248	5	955
Error[1]	118	990	105	369	113	568

[1]Error for testing Dam(Sire) was Sire MS.

***$P < .001$.

****$P < .0001$.

TABLE 5. Analyses of variance for percentage GAT bound by progeny from F_1 crosses including sire *Ea-B* (line) type

Source of variation	F_1 progeny crossed to S1 B^{19L} df	MS	F_1 progeny crossed to G-B1 B^{13} df	MS
Sire *Ea-B*	1	14,291***	1	88
Progeny *Ea-B*	1	4,452*	1	234,679***
Error	130	1,009	122	258

*$P < .05$.

***$P < .001$.

not present in the S1 line or were present at low frequency. This would not explain why progeny from F_1 chickens backcrossed to S1 B^{19L} chickens had the same GAT response as F_1 chickens backcrossed to G-B1 chickens. Perhaps crossing back to the S1 B^{19L} chickens dilutes the effects of the complementary genes and results in a lower GAT response than that of the progeny from crosses back to G-B1. Possibly, the F_2 birds maintained enough of the complementary alleles to result in high antibody response to GAT. Because high antibody response to antigen is invariably a dominant trait, the alleles would not have to be present in homozygous form to exert their effect.

Dunnington *et al.* (1989) found that the antibody response to SRBC was influenced by the MHC haplotype and the background genome of the chicken. Although SRBC represent a more complex antigen than GAT, the results of challenge would be dependent on the same immunological mechanisms. The antibody response to SRBC in S1 sublines was evaluated by Kim *et al.* (1987), and the resulting ANOVA indicated that neither *Ea-B* (as a marker for the MHC) nor Ir-GAT type was a significant source of variation but that their interaction was significant. Cheng and Lamont (1988) assayed the S1 line for immunocompetence and found significant differences based on prior selection for *Ea-B*, Ir-GAT, and response to Rous sarcoma virus-induced tumors. The interactions of some of these effects were also significant, indicating the involvement of other genetic controls.

The results reported in the current study illustrated that both MHC and non-MHC genes influenced humoral antibody response to GAT in the lines of chickens tested. Loudovaris *et al.* (1990) conducted a similar study in chickens but the lines they utilized were all low responders to GAT. They did find evidence of a single dominant gene linked to the MHC that determined high antibody response to the branched amino acid polymer (T,G)-A-L (tyrosine, glutamic acid-alanine-lysine). The lines of chickens used by Loudovaris *et al.* (1990), although highly inbred, were derived from one population pool. The chicken lines used in the present study were from unrelated backgrounds, which perhaps optimized the expression of additive gene effects to modulate antibody titer. Response or nonresponse may be determined by MHC genes, but the amount of antibody produced may be partially regulated by genes outside of the MHC.

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